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Flavonoids From Cressa Cretica

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فلافينويدات مستخلصة من كريسا كريتيريا

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تم إستخلاص خمسة أنواع مختلفة من الفلافينويد من جزء الأيريل لنبات كريسا كريتيريا L وتم تصنيفهم كما في المعادلة المرفقة إلى الفلافونويد رقم (1) و(2) و(3) و (4) و(5) وقد تمت دراسة هذه المركبات والتعرف عليها بواسطة أجهزة الطيف المختلفة مثل الأشعة الفوق بنفسجية وأجهزة الرنين النووي المغناطيسي (البروتون و الكربون 13) وأجهزة الكتلة FAB-MS وتعتبر الفلافينويد الأربعة 2-5 مركبات تم استخلاصها حديثا وتنشر لأول مرة من نبات كريسا كريتيريا L

Keywords: *Cressa cretica, Convolvulaceae, flavonoids.*

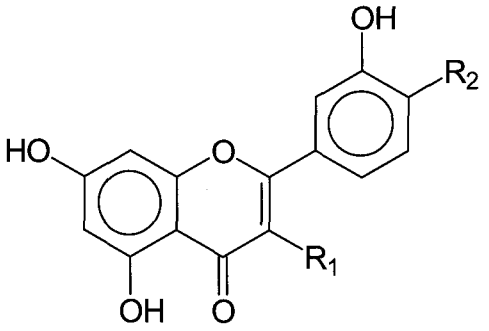
ABSTRACT

The aerial parts of *Cressa cretica* L. yielded five flavonoids that were identified as quercetin **(1)**, quercetin-3-β-O-D-glucoside **(2)**, kaempferol-3-O-β-D-glucoside **(3)**, kaempferol-3-O-α-L-rhamno-(1→6)- β-D-glucoside **(4)** and quercetin-3-O-α-L-rhamno-(1→6)- β-D-glucoside (rutin) **(5)**. All of the isolated flavonoids were identified by spectroscopic methods (UV, FAB-MS, ^{1}H-NMR and ^{13}C-NMR). The isolated flavonoids except quercetin are reported here for the first time from *Cressa cretica* L.

Introduction

Cressa cretica L. belongs to the Convolvulaceae family, known in Arabic as Molleih or Nadewa [1]. *C. cretica* is a remarkable salt tolerant plant, common in costal areas. *C. cretica* is mentioned as an Ayurevedic drug, known in indigenous medicine in India as Rudanti [2]. It is reported to be antibilious, antitubercular and expectorant [2,3]. The plant is used for alterative, anthelmintic, stomachic, tonic, and aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, asthma and urinary discharges [4]. The plant is traditionally used in Bahrain as expectorant and antibilious agent [2].

The phytochemical constituents of *Cressa critica* have not well been investigated. Only a few reports exist of the isolation of coumarins, sterols and quercetin glycosides (with unidentified sugars) [5]. Phytochemical screening of the plant growing in Qatar revealed the presence of alkaloids, coumarins and sterols [7]. The salt content of the plant is very high [6]. In a previous investigation the antiviral activity of different extracts and fractions from the plant was reported [8]. In view of the medicinal importance of *C. cretica* we undertook a systematic chemical investigation of the plant growing in Egypt.



	R1	R2
(1) quercetin	OH	OH
(2) kaempferol-3-O -β-D-glucoside	O-glu	H
(3) quercetin-3-O-β-D glucoside	O-glu	OH
(4) kaempferol-3-O-α-L-rhamnosyl-(1→6)-O-β-D-glucoside)	O-rut	H
(5) quercetin-3-O-α-L-rhamno-(1→6)- β-D-glucoside (rutin)	O-rut	OH

(rha = rhamnosyl; glu = glucosyl; rut = rutinosyl)

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General

FAB-MS spectral analysis in negative or positive mode was performed on a VG70-SEQ Hybrid Mass Spectrometer. All NMR spectra were run on a Bruker DRX-400 instrument. The chemical shifts were reported in δ values (ppm) with TMS as the internal standard. Carbon multiplicities were determined in DEPT-135 and DEPT-90 experiments. ^{1}H-and ^{13}C-NMR spectra were recorded

in CD_3OD. UV spectra were recorded on a UVIKON 931 double beam UV-VIS spectrophotometer in the region of 200-500nm. UV detection was at 254 and 336 nm. Thin layer chromatography was performed on Merck precoated Silica gel 60 F254 plates, while column chromatography was carried out using Merck silica gel 60 (230-400 mesh) as adsorbent. Solvent system for TLC was: ethyl acetate: acetic acid: formic acid: water (30:0.8:1.2:8) and the plates were sprayed by diphenyl boric acid – ethanolamine complex (Neu's spray reagent) [9].

Materials and Methods

Plant material

The whole plant of *Cressa cretica* L. was collected at Helwan, South Cairo, Egypt in October 1998 and identified by Dr. M. Elgebaly, Dept. of Chemotaxonomy, National Research Centre, Cairo, Egypt. A voucher specimen has been deposited at the Herbarium of the NRC, Cairo, Egypt.

Extraction and isolation

The air dried, powdered whole plant of *C. cretica* L. (1kg) was defatted with n-hexane and then extracted subsequently with $CHCl_3$, CH_2Cl_2/MeOH (1:1) and water. The CH_2Cl_2/MeOH extract was evaporated under reduced pressure and dissolved in aqueous MeOH 20%. The aqueous methanolic solution was partitioned first against $CHCl_3$, EtOAc and then against n-BuOH.

The EtOAc fraction was subjected to column chromatography (CC) on Sephadex LH-20 using propanol with increasing amounts (10%) of MeOH. Four fractions (Cr1-Cr4) were collected after monitoring with TLC. The spots were detected with UV before and after spraying. The fraction Cr2 was submitted to CC (Silica gel 60), the separation was initiated with $CHCl_3$ and polarity was gradually increased with steps of 5% of MeOH. The flavonoid-containing fraction was eluted with $CHCl_3$/MeOH (2:8) and then subjected to PTLC to give compounds **1-3**. The fraction eluted with $CHCl_3$/MeOH (3:7) was also subjected to PTLC using the same solvent followed by CC on Sephadex LH-20 and eluted with MeOH to give compounds **4** and **5**.

Experimental Data

Compound 1 (Quercetin)

Amorphous yellow solid, changing to orange on TLC with Neu's spray reagent, Rf= 0.93 negative FAB-MS $[M-H]^-$: *m/z* 301

UV λ_{max} (MeOH) 371, 257 nm, (NaOH) 424, 325 decompos., 287 nm, ($AlCl_3$) 445, 362, 272, 252 nm, ($AlCl_3$/HCl) 428, 340, 271 nm, (NaOAc) 388, 310 nm, (NaOAc/H_3BO_3) 386, 326, 262 nm.

Compound 2 (Kaempferol-3-*O*-β-D-glucoside)

A dull brown spot on the silica gel plate changing to orange with Neu's reagent.

Rf = 0.41, FAB-MS (negative ion mode): *m/z* 447 $[M-H]^-$, 285 $[M-H-162]^-$, UV λ_{max} (MeOH) 350, 318, 281 nm, (NaOH) 401, 346, 325 308 nm, ($AlCl_3$) 350, 318, 281 nm, ($AlCl_3$/HCl) 396, 375, 346 nm.

^1H –NMR (CD_3OD, 400 MHz): δ 8.02 (2H, d, *J*= 8.6 Hz, H-2', H-6'), 6.87 (2H, d, *J*= 8.6 Hz, H-3', H-5'), 6.40 (1H, s, H-8), 6.20 (1H, s, H-6), 5.10 (1H, s, H-1'')3.70-3.20 (5H, sugar). ^{13}C –NMR (CD_3OD, 100 MHz) δ. 159.25 (C-2), 135.54 (C-3), 179.52 (C-4), 163.01 (C-5), 100.03 (C-6), 166.25 (C-7), 94.85 (C-8), 158.54 (C-9), 105.68 (C-10), 122.73 (C-1'), 132.28 (C-2', C-6'), 116.11

(C-3', C-5'), 161.64 (C-4'), 104.29 (C-1''), 75.65 (C-2''), 78.37 (C-3''), 71.35 (C-4''); 78.01 (C-5''), 62.50 (C-6'').

Compound 3 (Quercetin-3- O-β-D-glucoside)

It was isolated as an amorphous brown solid changing to yellow with NH_3 and orange with Neu's reagent, Rf =0.32 (solvent B). The negative FAB-Mass spectrum showed a molecular ion $[M-H]^-$, at *m/z* 463 and 301. UV: λ_{max} (MeOH) 350, 282, 257 nm, (NaOH) 409, 350, 330, 272 nm, ($AlCl_3$) 430, 333, 275 nm, ($AlCl_3$/HCl) 403, 323, 270 nm (NaOAc) 381, 293, 264 nm, (NaOAc/H_3BO_3) 379, 319, 262.

^{1}H–NMR (CD_3OD, 400 MHz): δ 7.70 (H, d, *J*= 2.1 Hz, H-2'), 7.56 (H, dd, *J*= 8.4, 2.1 Hz, H-6'), 6.90 (1H, d, *J*= 8.4 Hz, H-5'), 6.32 (1H, d, *J*= 2.3 Hz, H-8), 6.15 (1H, d, *J*= 2.1 Hz, H-6), 5.12 (1H, d, *J*= 7.5 Hz, H-1'') 3.70-3.20 (5H, glucose).

^{13}C –NMR (CD_3OD, 100 MHz) δ158.40 (C-2), 135.60 (C-3), 179.40 (C-4), 163.00 (C-5), 99.80 (C-6), 166.00 (C-7), 94.70 (C-8), 159.00 (C-9), 105.70 (C-10), 123.10 (C1'), 117.50 (C-2'), 145.90 (C-3'), 149.80 (C4'), 116.00 (C5'), 123.20 (C6'), 104.30 (C-1''), 78.10 (C-2''), 75.70 (C-3''), 71.20 (C-4''), 78.3 0(C-5''), 62.50 (C-6'').

Compound 4 (Kaempferol-3-O-α-L-rhamnosyl-(1→6)-β-D-glucoside)

Rf = 0.12, FAB-MS (negative ion mode): 593 $[M-H]^-$, 447 $[M-H-146]^-$ 285 $[M-H-146-162]^-$

^{1}H –NMR (CD_3OD, 400 MHz): δ 8.09 (2H, d, *J*= 8.8 Hz, H-2', H-6'), 6.90 (2H, d, 8.8 Hz, H-3', H-5'), 6.42 (1H, s, H-8), 6.23 (1H, s, H-6) 5.10 (1H, d, *J*= 7.3 Hz H-1''), 4.53 (1H, a broad s, H-1'''),3.83-3.43 (10 H, rhamnose and glucose).

^{13}C –NMR (CD_3OD, 100 MHz): δ 179.40 (C-4), 166.71 (C-7), 162.99 (C-5), 161.60 (C-4'), 159.55 (C-9), 158.68 (C-2), 135.56 (C-3), 132.41 (C-2', C-6'), 122.81 (C-1'), 116.20 (C-3', C-5'), 104.70 (C-10), 102.48 (C-1'', C-1'''), 100.29 (C-6), 95.12 (C-8), 77.80 (C-3''), 77.20 (C-5''), 75.70 (C-2''), 73.9 (C-4'''), 72.30 (C-4''), 72.10 (C-2'''), 71.53 (C-3'''), 69.78 (C-5'''), 68.70 (C-6'') and 17.9 (C-6''')

Compound 5 (Quercetin-3-O-α-L-rhamno-(1→6)- β-D-glucoside) (rutin)

Crystalline yellow change to orange with Neu's reagent under UV.

Rf = 0.12, negative FAB-Mass spectrum $[M-H]^-$, at m/z 609, 301. UV λ_{max} (MeOH) 360, 284 nm, (NaOH) 412, 330, 330, 272 nm, ($AlCl_3$) 434, 320, 274 nm, ($AlCl_3$/HCl) 405, 315, 270 nm (NaOAc) 382, 317, 264 nm, (NaOAc/H_3BO_3) 382, 320, 264.

Results and Discussion

For Compound 1 (Quercetin) colour reactions (yellow, changing to orange with Neu's reagent) indicated the presence of a 3', 4' dihydroxyl substitution in the B-ring [10]. Chromatographic behaviour and UV spectral analysis with the usual shift reagents indicated the presence of free hydroxyl groups at C-3, C-5 and C-4'. The absorption peak at 371 nm was characteristic for flavonols (3-OH free).

The pseudo molecular ion of compound **1** in positive FAB-mass spectrum $[M+H]^+$ at *m/z* 303 indicated a molecular weight of 302, which is consistent with quercetin. Finally the identity of quercetin was established by direct co-chromatography with an authentic sample.

For compound 2, the UV absorbance at 350 nm in MeOH indicated a 3-O-substituted flavonol [11]. The bathochromic shift on addition of $AlCl_3$ and NaOH indicated the presence of free OH

groups at C-5 and C-4' respectively. The four aromatic protons at δ 8.02 and δ 6.87 as shown in the ^{1}H- NMR spectrum were assigned to H-2' / H-6' and H-3'/H-5' and the two doublets at δ6.80 and 6.36 assigned to H-8 and H-6 respectively. ^{13}C-NMR showed 15 signals that could be attributed to the aglycone and 6 signals that were due to the sugar moiety. The DEPT experiments showed these signals to be 1 CH_2, 11 CH and 9 quaternary carbons. The anomeric proton (H-1'') of the hexose appeared as a doublet at δ 5.18 (J=7.2 Hz). This chemical shift confirmed that glucose is attached to the aglycone moiety and the diaxial coupling J=7.2Hz between H-1 and H-2 indicated that the hexose had a β-configuration [10].

The FAB-Mass spectrum displayed a $[M-H]^-$ ion at *m/z* 447 corresponding to the deporotonated molecule. Another significant peak was visible at *m/z* 285 $[M-H-162]^-$, due to the loss of a hexose residue. Acid hydrolysis of compound **2** afforded glucose (co-TLC) and an aglycone, which was identified as kaempferol by comparison of TLC, UV and FAB-MS data with literature values. According to these data compound **2** was identified as **kaempferol-3-O-β-D-glucoside.**

A dull brown spot on the silica gel plate changing to orange with Neu's reagent and UV analysis in the presence of the usual shift reagent suggested that compound **3** was a 3-substituted flavonol.

The UV spectrum in methanol (354 nm, band I) indicated a flavonol 3-O-substituted flavonol. The bathochromic shift (56 nm) on addition of NaOH without decrease of intensity confirmed the presence of a free 4'-OH. The presence of a 7-OH group was indicated by the bathochromic shift of 11 nm with NaOAc (band II). The bathochromic shift (25 nm band I) with NaOAc/ H_3BO_3 suggested the presence of o-dihydroxy groups in the B-ring. The 5-OH group was confirmed by the bathochromic shift of band I with $AlCl_3$/HCl (41 nm). These data were confirmed by ^{1}H and ^{13}C-NMR.

The ^{1}H and ^{13}C-NMR spectra indicated the presence of a quercetin moiety and sugar unit (Markham and Chari, 1982). The doublet at δ 5.33 (diaxial coupling J=7.5Hz) was assigned to the anomeric proton of hexose and suggested a glycosidic β-linkage. FAB-mass spectrum revealed signals at *m/z* 463 and *m/z* 301, which were ascribed to $[M-H]^-$ and $[M-H-162]^-$ ions respectively, suggesting one hexose moiety in the molecule. Acid hydrolysis of compound **3** gave glucose and quercetin, which were identified by co-chromatography with an authentic sample. On the basis of these data compound **3** was identified as **quercetin-3- O-β-D-glucoside.**

The UV spectrum of compound **4** in MeOH showed the same absorption peaks as compound **2**, indicating it is a flavonol glycoside.

The negative FAB-Mass spectrum showed a deprotonated molecule at m/z 593 $[M-H]^-$, *m/z* 477 $[M-H-146]^-$, and *m/z* 285 $[M-H-146-162]^-$ signals indicated the successive elimination of rhamnose and glucose respectively and supported a 3-glycosylated flavonol structure with rhamnose as the terminal unit. The sugars and the aglycone were identified after acid hydrolysis as kaempferol, rhamnose and glucose by co-TLC with reference samples. The interglycosidic linkage was determined by ^{1}H and ^{13}C-NMR spectroscopy. The ^{13}C-NMR chemical shift of C-2 was observed downfield (δ 158.68) compared with C-2 of kaempferol indicating 3-glycosylation (Markham and Chari, 1982). The interglycosidic (1→6) linkage was confirmed by the downfield shift of C-6 of the glucose moiety by 6.2 ppm relative to an unsubstituted glucose moiety (C-6 at δ 62.5). The protons at position 6 and 8 occurred at δ 6.23 and 6.40, respectively, in ^{1}H-NMR and at δ 100.29 and 95.12 respectively, in ^{13}C-NMR. The two ortho coupled doublets at δ 6.90 and 8.09 (J = 8.8 Hz) were assigned to H-3'/H-5' and H-2'/ H-6', respectively. The corresponding carbons were found at δ 116.2 and 132.4 respectively. The anomeric protons at δ 5.1 (d, J=7.3 Hz) and 4.5 (broad s) were assigned to H-1 of β-glucose and α-rhamnose for which the corresponding carbons resonated at δ

104.71 and 102.48, respectively. According to these data compound **4** was identified as **(Kaempferol-3-O-α-L rhamnosyl-(1→6)-β-D-glucoside.**

Compound 5 was identified as rutin from the UV spectral data and negative FAB-MS. UV spectra suggested an *o*-dihydroxy flavonol substituted at position 3. The free hydroxyl groups at position 5, 7 and 4' were deduced from the bathochromic shift of band I with $AlCl_3$/HCl, of band II with NaOAc and band I with NaOH. The FAB-mass spectrum (negative mode) showed signals at *m/z* 609 $[M-H]^-$, and at *m/z* 301 $[M-H-308]^-$ indicating combined loss of glucose and rhamnose. Acid hydrolysis of compound V-7 afforded quercetin, glucose and rhamnose.

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